Claims

1. Use of one or more S3P primers in a method for analysing or amplifying a nucleic acid sequence.

2. Use according to claim 1, in combination with at least one AFLP primer.

3. Use according to claim 1 or 2 wherein the nucleic acid sequence comprises a restriction fragment to which one, preferably two adapter sequences have been ligated.

4. Use according to claim 3, in which the restriction fragment to which the adapter sequence has been ligated is part of a mixture of adapter-ligated restriction fragments.

5. Use according to any of the claims 1-4, in which the nucleic acid sequence, and in particular the restriction fragment, contains or is suspected to contain, an intron-exon junction and/or a splice site.

6. Use according to any of the claims 1-5, in which the restriction fragment is derived from genomic DNA, mitochondrial DNA, chloroplast DNA, recombinant DNA or unprocessed heteronuclear mRNA.

7. Use according to any of the claims 1-6, wherein the S3P primer is in an intron-to-exon orientation or in an exon-to-intron orientation.

8. Use according to any of the claims 2-7, wherein the AFLP primer contains at least one selective nucleotide at its 3' end.

9. Use according to claims 1-8, wherein the S3P primer is a primer comprising a conserved splice site border sequence or at least part of a consensus sequence, preferably at least 50 % of the consensus sequence, more preferably at least 60 %, 70%, 80% ,or 90%, most preferably 100% of the consensus sequence.

10. Use according to claim 9, wherein the S3P primer further comprises a random sequence.

11. Use according to any of the claims 1-10, wherein S3P primer is specific for a splice site selected from the group consisting of GU-AG introns, AU-AC introns, Group I introns, Group II introns, Group III introns, Twintrons, Pre-tRNA introns, and splice sites that are identified using computer based splice site identification methods.

12. Use according to any of claims 1-11, wherein the S3P primer contains a total of between 8 and 20 nucleotides, preferably between 12 and 16 nucleotides.

13. Use according to any of claims 1-12, wherein between 4 and 10, preferably between 6 and 8 nucleotides present in the S3P-primer are complementary to the conserved region or consensus sequence of the splice site.

14. Use according to any of the claims 1-13, wherein the consensus sequence is $X_1X_2GTX_3X_4X_5X_6$, wherein X_1, X_2, X_3, X_4, X_5, X_6 are independently selected from the group consisting of A,C,T, or G.

15. Use according to claim 14, wherein the consensus sequence is AGGTAAGT.

16. Method for analyzing a nucleic acid sequence, the method at least comprising the steps of:
(a) amplifying an adapter-ligated restriction fragment generated from the nucleic acid to be analysed, using one or more S3P-primers and optionally an AFLP-primer, whereby each of the S3P and the AFLP primer are as defined in any of the claims 1-2 and 7-15, to amplify the nucleic acid sequence; and optionally comprising the further step of:
(b) detecting the amplified nucleic acid sequences thus obtained.

17. Method for analyzing a nucleic acid sequence, the method comprising the steps of:
(a) restricting the starting nucleic acid with a restriction endonuclease to provide a

mixture of restriction fragments;

(b) ligating the restriction fragments thus obtained to at least one adapter;

(c) amplifying the mixture of adapter-ligated restriction fragments thus obtained with one or more S3P-primers and optionally at least one AFLP-primer, whereby each of the S3P and the AFLP primer are as defined in any of the claims 1-2 and 7-15, to provide a mixture of amplified restriction fragments; and optionally comprising the further step of

(d) detecting the amplified restriction fragments thus obtained.

18. Method for the amplification of at least one restriction fragment obtained from a starting DNA, comprising the steps of :

(a) digesting the starting DNA with at least one restriction endonuclease, thereby providing one or more restriction fragments;

(b) ligating at least one oligonucleotide adapter to one or both ends of the restriction fragments to provide adapter-ligated restriction fragments;

(c) providing a primer set comprising one or more S3P primers and optionally at least one AFLP primer, whereby each of the S3P and the AFLP primer are as defined in any of the claims 1-2 and 7-15;

(d) contacting the adapter-ligated restriction fragments with the set of primers;

(e) amplifying the adapter-ligated restriction fragments with the set of primers; and

(f) recovery of any amplified DNA fragments.

19. Method for providing a PCR primer or a pair of PCR primers for use in the amplification of a PCR fragment spanning a splice site-associated genomic polymorphism, the method comprising the steps of:

a) identification of a fragment containing the splice site-associated genomic polymorphism, whereby the fragment is amplified by the combined use of one or more S3P primers and optionally at least one first AFLP primer, whereby each of the S3P and the AFLP primer are as defined in any of the claims 1-2 and 7-15, for a first restriction enzyme used for AFLP template preparation;

b) sequencing the polymorphic fragment;

c) synthesizing a first PCR-primer corresponding to a sequence flanking the splice site sequence at the 3' end;

d) optionally, amplifying a fragment comprising the splice site-associated genomic polymorphism and sequences flanking the splice site-associated genomic polymorphism at its 5'-end, using the first PCR-primer and a second AFLP primer for a second restriction enzyme used for AFLP template preparation; and,

e) optionally, synthesizing a second PCR-primer corresponding to a sequence flanking the splice site sequence at the 5'end.

20. Method for providing a PCR-primer, comprising the steps of:

a) restricting a nucleic acid sequence with at least one restriction endonuclease to provide a mixture of restriction fragments;

b) ligating the restriction fragments thus obtained to at least one adapter;

c) amplifying the mixture of adapter ligated restriction fragments thus obtained with at least one S3P-primer and optionally at least one first AFLP-primer, whereby each of the S3P and the AFLP primer are as defined in any of the claims 1-2 and 7-15, to provide a mixture of amplified restriction fragments;

d) detecting at least one of the amplified restriction fragments thus obtained;

e) identifying at least one splice site-associated polymorphic fragment;

f) determining the sequence of said polymorphic fragment;

g) synthesizing a first PCR-primer corresponding to a sequence flanking the splice site sequence at the 3' end;

h) optionally, amplifying a fragment comprising the splice site and at least part of the 5'-flanking sequence using the first PCR-primer and a second AFLP primer used in AFLP template preparation; and

i) optionally, synthesizing a first PCR-primer corresponding to a sequence flanking the splice site sequence at the 5'end.

21. Use of a PCR-primer obtainable by the method of claims 15 or 16 in the development of an assay, preferably for the analysis of splice sites.

22. Use of (the combination of) one or more S3P primers and optionally at least one AFLP primer in the development of PCR-primers.

23. Kit comprising means for performing a method as defined in any one of claims 7-14, preferably comprising at least one S3P primer and optionally at least one AFLP primer, optionally in combination with other kit components per se.

24. Kit comprising PCR-primers obtained by the method of claim 15 or 16.

25. Use of the method according to claim 18 for the (selective) enrichment of a sample for nuclear or organelle derived amplification products.